

Amendment and Response

Applicants: Sprecher et al.

Serial No.: 10/800,057

Filed: March 12, 2004

For: NOVEL HUMAN KUNITZ-TYPE INHIBITORS AND METHODS RELATING THERETO

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In the Claims

Per 37 C.F.R. §1.121, the current status of all the claims in the present application is presented below, amended claims are noted to indicated changes made and the text of pending claims not being amended are presented clean. Amendments to the following are indicated by underlining what has been added and striking-through what has been deleted.

This listing of the claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): An isolated human Kunitz-type inhibitor that inhibits plasmin in a mammal and, wherein a DNA sequence encoding the human Kunitz-type inhibitor hybridizes to nucleotides 138-305 of SEQ ID NO:1 under highly stringent hybridization conditions of 6x SSC, 5x Denhardt's reagent, 0.5% SDS, and 100ug/ml denatured and fragmented salmon sperm DNA hybridized at 68°C for about 12-16 hours and washed in 2x SSC, 0.5% SDS at room temperature for about 5 minutes, followed by a wash in 2x SSC, 0.1% SDS at room temperature for about 15 minutes, followed by a wash in 0.1x SSC, 0.5% SDS at 37°C for about 0.5-1 hour, followed by a wash in 0.1x SSC, 0.5% SDS at 68°C for about 0.5-1 hour, followed by a wash in 0.1x SSC at room temperature for about 3-5 minutes.

Claim 2 (canceled)

Claim 3 (previously presented): A pharmaceutical composition comprising the human Kunitz-type inhibitor of claim 1.

Claim 4 (previously presented): The pharmaceutical composition of claim 3 wherein the human Kunitz-type inhibitor is isolated from *E. coli*.

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Claim 5 (previously presented): A DNA construct comprising a first DNA segment, wherein the first DNA segment is the DNA sequence of claim 1, operably linked to additional DNA segments required for the expression of the first DNA segment.

Claim 6 (previously presented): A host cell comprising the DNA construct of claim 5 wherein the host cell expresses the human Kunitz-type inhibitor encoded by the first DNA segment.

Claim 7 (previously presented): The host cell of claim 6 wherein the host cell is *E. coli*.

Claim 8 (previously presented): A method for producing human Kunitz-type inhibitor comprising:

culturing a cell according to claim 6; and
isolating the human Kunitz-type inhibitor produced by the cell.

Claim 9 (previously presented): The method of claim 8 wherein the cell is *E. coli*.

Claim 10 (currently amended): An isolated DNA sequence that hybridizes to nucleotides 138-305 SEQ ID NO:1 under highly stringent hybridization conditions of 6x SSC, 5x Denhardt's reagent, 0.5% SDS, and 100µg/ml denatured and fragmented salmon sperm DNA hybridized at 68°C for about 12-16 hours and washed in 2x SSC, 0.5% SDS at room temperature for about 5 minutes, followed by a wash in 2x SSC, 0.1% SDS at room temperature for about 15 minutes, followed by a wash in 0.1x SSC, 0.5% SDS at 37°C for about 0.5-1 hour, followed by a wash in 0.1x SSC, 0.5% SDS at 68°C for about 0.5-1 hour, followed by a wash in 0.1x SSC at room temperature for about 3-5 minutes, wherein the isolated DNA sequence encodes a human Kunitz-type inhibitor that inhibits plasmin in a mammal.

Claim 11 (canceled)

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Claim 12 (currently amended): An isolated human Kunitz-type inhibitor that inhibits plasmin in a mammal and, wherein a DNA sequence encoding the human Kunitz-type inhibitor hybridizes to nucleotides 39-743 of SEQ ID NO:1 under highly stringent hybridization conditions of 6x SSC, 5x Denhardt's reagent, 0.5% SDS, and 100µg/ml denatured and fragmented salmon sperm DNA hybridized at 68°C for about 12-16 hours and washed in 2x SSC, 0.5% SDS at room temperature for about 5 minutes, followed by a wash in 2x SSC, 0.1% SDS at room temperature for about 15 minutes, followed by a wash in 0.1x SSC, 0.5% SDS at 37°C for about 0.5-1 hour, followed by a wash in 0.1x SSC, 0.5% SDS at 68°C for about 0.5-1 hour, followed by a wash in 0.1x SSC at room temperature for about 3-5 minutes.

Claim 13 (canceled)

Claim 14 (previously presented): A pharmaceutical composition comprising the human Kunitz-type inhibitor of claim 12.

Claim 15 (previously presented): The pharmaceutical composition of claim 14 wherein the human Kunitz-type inhibitor is isolated from *E. coli*.

Claim 16 (previously presented): A DNA construct comprising a first DNA segment, wherein the first DNA segment is the DNA sequence of claim 12, operably linked to additional DNA segments required for the expression of the first DNA segment.

Claim 17 (previously presented): A host cell comprising the DNA construct of claim 16 wherein the host cell expresses the human Kunitz-type inhibitor encoded by the first DNA segment.

Claim 18 (previously presented): The host cell of claim 17 wherein the host cell is *E. coli*.

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Claim 19 (previously presented): A method for producing human Kunitz-type inhibitor comprising:

culturing; a cell according to claim 17; and
isolating the human Kunitz-type inhibitor produced by the cell.

Claim 20 (previously presented): The method of claim 19 wherein the cell is *E. coli*.

Claim 21 (currently amended): An isolated DNA sequence that hybridizes to nucleotides 39-743 SEQ ID NO:1 under highly stringent hybridization conditions of 6x SSC, 5x Denhardt's reagent, 0.5% SDS, and 100 μ g/ml denatured and fragmented salmon sperm DNA hybridized at 68°C for about 12-16 hours and washed in 2x SSC, 0.5% SDS at room temperature for about 5 minutes, followed by a wash in 2x SSC, 0.1% SDS at room temperature for about 15 minutes, followed by a wash in 0.1x SSC, 0.5% SDS at 37°C for about 0.5-1 hour, followed by a wash in 0.1x SSC, 0.5% SDS at 68°C for about 0.5-1 hour, followed by a wash in 0.1x SSC at room temperature for about 3-5 minutes, wherein the isolated DNA sequence encodes a human Kunitz-type inhibitor that inhibits plasmin in a mammal.

Claim 22 (previously presented): The isolated DNA sequence of claim 21 wherein any differences between the encoded human Kunitz-type inhibitor and amino acid number 1 to amino acid number 235 of SEQ ID NO:2 are due to conservative amino acid substitutions.